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Correlation between time to positive result of SARS-CoV-2 rapid antigen self-test and viral antigen concentration

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ABSTRACT

Background

This study was planned to investigate how the positivization time of a SARS-CoV-2 rapid antigen self-test may correlate with SARS-CoV-2 nucleocapsid (N) antigen concentration measured with a quantitative laboratory-based immunoassay.

Methods

Paired nasopharyngeal (healthcare-collected) and nasal (self-collected) samples were taken from patients undergoing routine SARS-CoV-2 testing. The concentration of SARS-CoV-2 antigen nucleocapsid (N) was assayed with Liaison SARS-CoV-2 Antigen test, whilst the time of positivization of COVID-VIRO ALL rapid diagnostic test (RDT) was concomitantly measured and then compared SARS-CoV-2 viral load measured with Liaison SARS-CoV-2 Antigen test and expressed as Median Tissue Culture Infectious Dose (TCID50)/mL.

Results

The study sample consisted of 32 paired specimens which tested positive with COVID-VIRO ALL IN RDT and had SARS-CoV-2 N protein concentration measured with Liaison SARS-CoV-2 Antigen test. A highly significant correlation was found between SARS-CoV-2 viral antigen concentration and RDT positivization time (r=-0.64; 95%CI, -0.81 to -0.38; p<0.001). At the >1500 TCID50/mL threshold of the Liaison SARS-CoV-2 Antigen test, the positivization time of the COVID-VIRO ALL IN RDT displayed high accuracy (93.7%). A positivization time <42 sec enabled to identify patients with high SARS-CoV-2 antigen concentration (i.e., >1500 TCID50/mL) with 91.3% negative and 100% positive predictive values.

Conclusion

Self-testing using COVID-VIRO ALL IN RDT could be reliably used for garnering valuable information on the actual SARS-CoV-2 viral antigen concentration in respiratory samples.

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INTRODUCTION

The continued development and availability of fast, decentralized, relatively inexpensive and accurate severe acute respiratory syndrome coronavirus disease 2 (SARS-CoV-2) rapid diagnostic tests (RDTs) is imperative, since the volume of routine and urgent molecular tests that need to be performed all around the world largely outweigh the current capacity of most clinical laboratories. An ongoing worldwide survey promoted by the American Association for Clinical Chemistry (AACC), aimed at defining the state-of-the-art of the coronavirus disease 2019 (COVID-19) testing capacity, highlights that over two-third of responding laboratories are still facing problems in obtaining enough reagents and test kits for routine diagnosis of SARS-CoV-2 infection [1], thus paving the way to planning and validating alternative strategies that may overcome the bottleneck caused by the relatively long turnaround time and low throughput of molecular testing. Recent guidelines and recommendations, such as those of the World Health Organization (WHO) [2] and International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) [3], have endorsed the possible use of SARS-CoV-2 RDTs under specific circumstance, such as for population screening (i.e., before large mass gatherings or prior to accessing healthcare facilities) and epidemiological purposes. One major and well-recognized limitation of these tests, along with their lower diagnostic accuracy [4], is represented by the generation of qualitative test results (i.e., negative or positive), which would then encumber the possibility to obtain information on SARS-CoV-2 viral load, a useful parameter for predicting infectivity, monitoring the course of disease and stratifying the risk of unfavourable disease progression [5]. Nonetheless, interesting evidence is emerging that the time to positive reaction of RDTs may be used for roughly predicting the SARS-CoV-2 viral load (i.e., the faster the positivization time, the higher the viral load) [6,7]. To this end, we planned this study to verify how the positivization time of a SARS-CoV-2 rapid antigen self-test may correlate with SARS-CoV-2 nucleocapsid (N) antigen concentration measured with a quantitative laboratory-based immunoassay.

MATERIALS AND METHODS

Study population

The study population consisted of a series of outpatients presenting to the Pederzoli Hospital in Peschiera del Garda (Verona, Italy) for undergoing routine SARS-CoV-2 testing between August 2 and September 3, 2022, when SARS-CoV-2 Omicron BA.5 prevalence was >90%. A

nasopharyngeal (healthcare-collected; Virus swab UTM Copan, Brescia, Italy) and nasal (self-collected; COVID-VIRO ALL) samples were taken upon patient admission, the former for being assayed in the local laboratory with Liaison SARS-CoV-2 Antigen test, the latter for performing COVID-VIRO ALL IN RDT, thus allowing faster screening of patients upon hospital presentation. All patients were instructed to correctly use the self-device by reading a quick utilization notice [8].

DiaSorin Liaison SARS-CoV-2 Antigen test

The immunochemical detection of SARS-CoV-2 in nasopharyngeal samples was carried out using DiaSorin Liaison SARS-CoV-2 Antigen test (DiaSorin, Saluggia, Italy), a fully-automated chemiluminescence sandwich-immunoassay (CLIA) that specifically developed for detecting SARS-CoV-2 nucleocapsid (N) protein in nasal and nasopharyngeal swabs, as described in details elsewhere [9]. The test, locally adapted for use on a DiaSorin LIAISON XL immunochemistry platform, displays an analytical sensitivity (i.e., limit of detection [LOD]) and a diagnostic threshold of 22.0 and 200 Median Tissue Culture Infectious Dose ($TCID_{50}$)/mL, respectively. A recent clinical investigation, assessing the cumulative diagnostic performance of this immunoassay during a period of SARS-CoV-2 Omicron predominance revealed that the diagnostic sensitivity and specificity were 0.93 and 1.00, respectively [10].

COVID-VIRO ALL IN RDT

COVID-VIRO ALL IN (AAZ-LMB, Boulogne-Billancourt, France) is a vertical flow immunoassay based on immunochemical detection of SARS-CoV-2 core (C) antigen in nasal specimens. The device could be used by healthcare professionals but is also suitable for self-testing due to its relatively simplicity of use, as comprehensively described elsewhere [11]. Briefly, after inserting the soft sponge at the upper part of the device in each nostril for 15 sec, the test kit is directly activated by pressing firmly the bottom of the holder (and hence without the need to twist the specimen in the reaction buffer and applying drops of the sample to the device, as for most RDTs), which breaks the buffer capsule and starts the reaction. After 15 min, the presence of two-coloured bands in the control (C) and test (T) windows reflects test positivity, their combined absence mirrors test failure, while the presence of a single coloured band in the control (C) windows defined test negativity. The testing procedure typically takes around 1 min and test results are within 15 min. According to data published in a recent clinical assessment this test, the positive predictive value (PPV) and negative predictive value (NPV) were 100% and 96.2%, respectively [11]. For this specific study, the positivization time of the RDT was measured with a manual chronometer by a healthcare professional, defined as the period between device activation to appearance of a coloured band in the test (T) windows, always accompanied by simultaneous presence of a coloured band in the (C) control window.

Statistical analysis

Test results were finally reported as median values and interquartile range (IQR). The agreement between antigen nucleocapsid concentration measured with Liaison SARS-CoV-2 Antigen test and time of positivization of COVID-VIRO ALL RDT was analyzed using Spearman's correlation and receiver operating characteristic (ROC) curve analysis. Since the test results displayed a non-normal distribution as assessed by Shapiro-Wilk test, they were transformed using natural logarithms before being analyzed. The correlation between the two measures could obviously only be conducted using COVID-VIRO ALL IN RDT positive samples, in which a numeric value of positivization time could be measured. The following statistical analysis was carried out

with Analyse-it software (Analyse-it Software Ltd, Leeds, UK). The study was conducted in accordance with the Declaration of Helsinki, under the terms of relevant local legislation, and was part of larger study protocol previously approved by the Ethical Committee of Verona and Rovigo Provinces (971CESC; Approved July 25, 2016).

RESULTS

The study sample consisted of 32 consecutive paired specimens which tested positive with COVID-VIRO ALL IN RDT (appearance of two bands in the control and test windows, respectively) and having also SARS-CoV-2 N protein concentration measured with Liaison SARS-CoV-2 Antigen test (median age 44 years, IQR 35-52 years; 75% women). The median SARS-CoV-2 viral antigen concentration was 1006 (IQR, 339-7169) TCID50/mL, whilst the median time of positivization of COVID-VIRO ALL IN RDT was 64 (IQR, 35-156) sec. No correlation was found between presence of symptoms (n=20) and TCID50/mL values (r=0.01; 95%CI, -0.34 to 0.36; p=0.940).

The association between SARS-CoV-2 viral antigen concentration and RDT positivization time is shown in Figure 1, evidencing a highly significant inverse correlation between these two measures (r= -0.64; 95%CI, -0.81 to -0.38; p<0.001).

At the >1500 $TCID_{50}$/mL threshold of Liaison SARS-CoV-2 Antigen test, which was earlier shown to reflect high viral antigen concentration and thereby greater risk of both infectivity and unfavourable clinical outcomes [12,13.], the diagnostic accuracy of COVID-VIRO ALL IN RDT positivization time was 93.7% (95%CI, 79.2 to 99.2%), with an area under the curve (AUC) of 0.88 (95%CI, 0.71 to 1.00; p<0.001) (Figure 2).

The best cut-off for predicting SARS-CoV-2 viral antigen concentration >1500 TCID50/mL was <42 sec of RDT positivization, which was associated with 91.3% (95%CI, 75.0-97.4%) NPV and 100% (95%CI, 100-100%) PPV, respectively.

DISCUSSION

Several lines of evidence now attest that the use of SARS-CoV-2 RDTs may represent a potential solution to overcome the current shortage of technical (and even human) resources needed as the COVID-19 pandemic progresses unremittingly [14]. The surge of SARS-CoV-2 infections sustained by recent and highly mutated lineages, especially BA.4/5 and BA.2.75 [15], is imposing a dramatic pressure on medical laboratories and other testing facilities, thus persuading several governments and health organizations worldwide to endorse the use of decentralized self-testing for widespread community testing as well as for optimizing the length of quarantine and/or isolation [16,17].

The generation of qualitative data, in terms of negative or positive test results, which are usually reflected by absence or presence of a colored band in the test window of the device, is a widely recognized shortcoming of RDTs, which does not implicitly consent to garner information on the actual viral load expressed by positive subjects. A tentative solution to this limitation has been provided by two preliminary investigations. Akashi et al. measured the positivization time of the QuickNavi™-COVID19 Ag RDT in 84 consecutive patient nasopharyngeal samples [6], and found a linear association between the viral load (i.e., cycle threshold values of SARS-Cov-2 N2 gene) and time to achieve a positive result (p< 0.001). Predictably, the positivization time of the RDT was longer in samples bearing a high viral load (cycle threshold values ≤31). In a following investigation, Salvagno et al. measured the positivization time of Roche SARS-CoV-2 Rapid Antigen Test in 106 patients with SARS-CoV-2 infection [7], and also found a significant correlation between the cycle thresholds values

of SARS-CoV-2 E and S genes and the RDT positivization time (r= 0.70; p<0.001), displaying an overall agreement of nearly 71% for identifying samples with high viral load (i.e., cycle thresholds values <20).

Taken together, the results of the present investigation support and extended the validity of these earlier findings, using a different SARS-CoV-2 RDT (most suited to be used as a self-test), self-administered, and in a period characterized by high prevalence of the SARS-CoV-2 Omicron BA.5 lineage.

In brief, we confirmed that SARS-CoV-2 viral antigen concentration and RDT positivization time obtained with self-testing are highly inversely correlated (i.e., r= -0.64), such that the sooner the coloured band will appears in the test window, the higher is the viral load expressed

Figure 1 **Spearman's correlation between positivization time of COVID VIRO ALL IN rapid diagnostic test (RDT) and viral load expressed as Median Tissue Culture Infectious Dose (TCID50)/mL and measured with DiaSorin Liaison SARS-CoV-2 Antigen test**

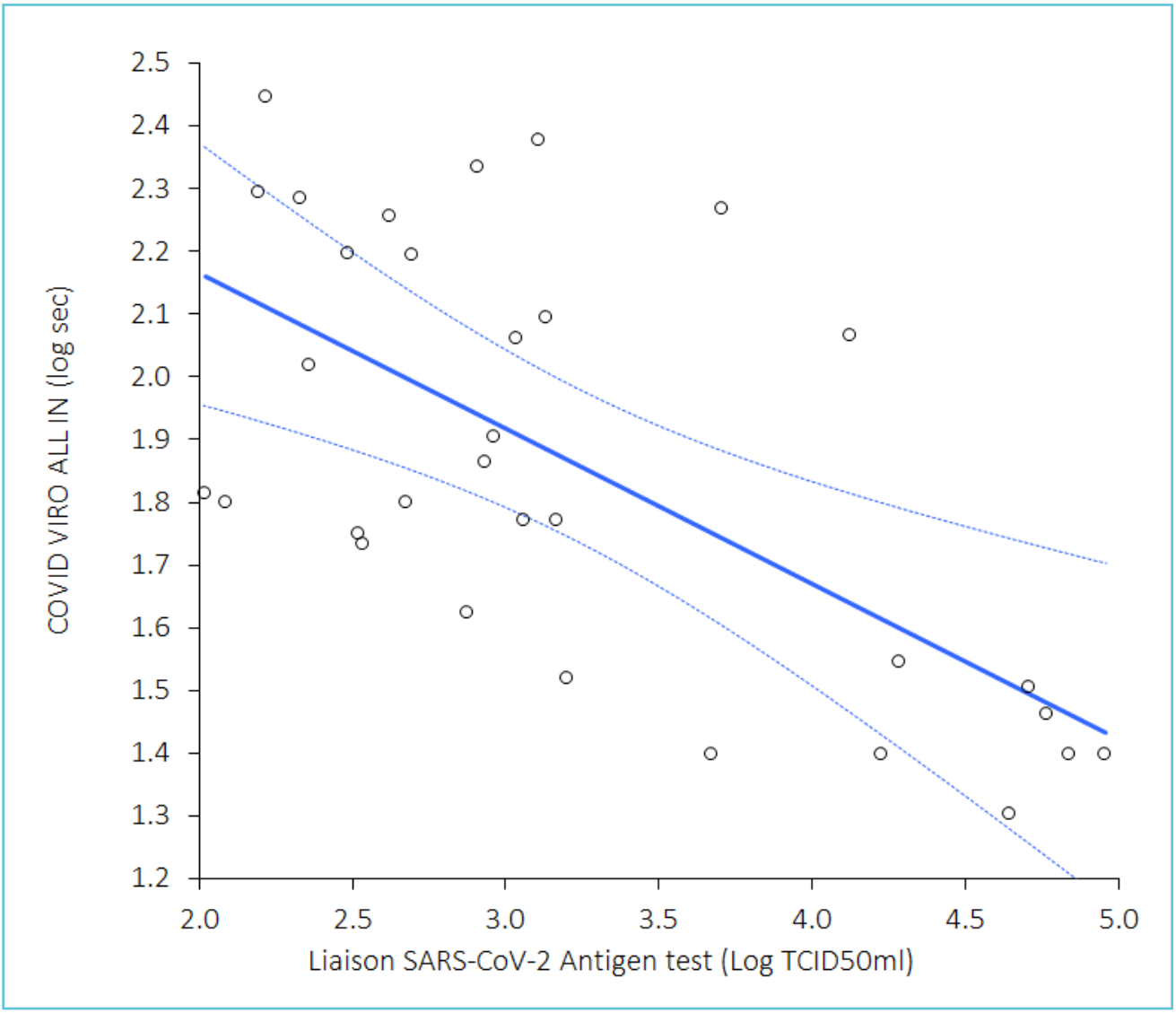
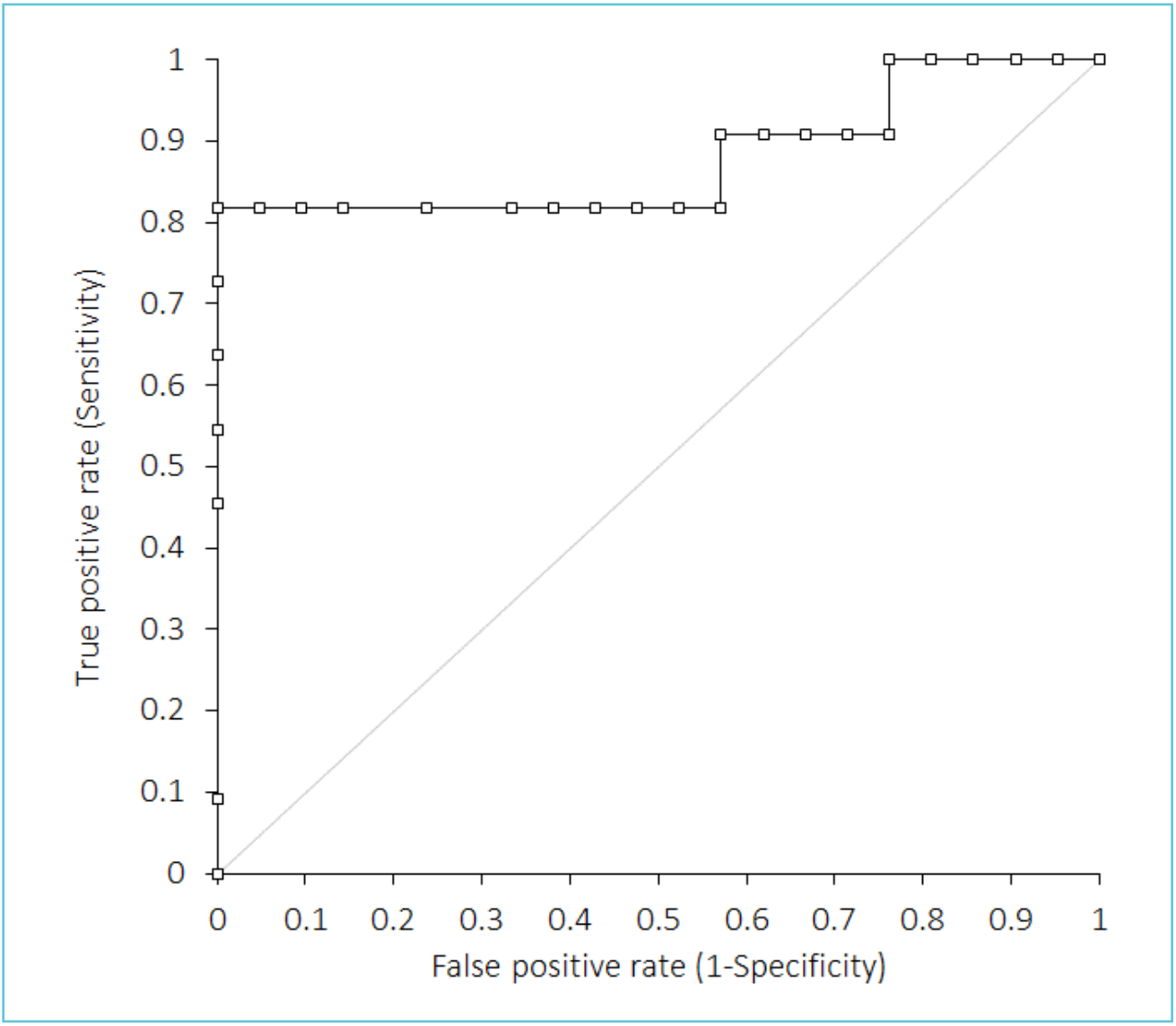


Figure 2 **Receiver operating characteristics (ROC) curve analysis of positivization time of COVID VIRO ALL IN rapid diagnostic test (RDT) for identifying samples with >1500 SARS-CoV-2 Median Tissue Culture Infectious Dose ($TCID_{50}$)/mL**



as SARS-CoV-2 N protein concentration. We also found that setting a positivization time of this device at <42 sec may enable identification of patients with high viral antigen concentration (i.e., >1500 $TCID_{50}$/mL) with over 90% NPV and 100% PPV, respectively. Interestingly, we also noticed that the IQR of COVID VIRO RDT positivization was much shorter (i.e., 35-156 sec) that the time window suggested by the manufacturer for test result availability (i.e., 15 min). This is a general aspect of all SARS-CoV-2 flow lateral immunoassays, in that the time window for reading results provided by manufacturers is typically longer than the effective time of positivization. This is probably due to legal reasons, for ensuring that the patients will wait a sufficient amount of time before reading the final test results.

CONCLUSIONS

The evidence that emerged from this study, combined with earlier published data which demonstrated that negativity or positivity of SARS-CoV-2 antigen tests may reflect the absence or presence of replication-competent virus [18], ultimately suggests that measuring the positivization time of the novel and user-friendly COVID-VIRO ALL IN RDT could be used for garnering valuable information on the actual SARS-CoV-2 viral RNA [19] and antigen concentration, even outside a specific healthcare setting, and thus providing a possible solution to relief the high workload currently caused by the ongoing COVID-19 pandemic [20].

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Conflicts of interest

The authors declare no conflict of interest.

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